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13. ABSTRACT *(Maximum 200 words)*

Levels of the eIF-2α-specific protein kinase, PKR, are higher in several breast carcinoma cell lines including the estrogen-responsive cell lines, MCF-7 and T-47D, as well as estrogen-independent cell lines, BT-20 and MDA-MB-468, compared with the normal breast cell line, Hs578 Bst, or the human HeLa cell line. In contrast, the phosphorylation state of eIF-2α is very low in the breast carcinoma cell lines compared to the normal human breast cell line Hs578 Bst and HeLa cells, even at high cell densities, suggesting an inhibition of PKR activity in the breast carcinoma cell lines. In support of this hypothesis, treatment of cells with either α- or β-interferon, although increasing PKR levels slightly, do not result in higher steady state levels of eIF-2α phosphorylation.

These results suggest that deregulation of PKR activity is occurring at some level in breast carcinoma cell lines. This deregulation could result from increased levels/activities of cellular PKR inhibitory proteins, or from inactivating mutations in the PKR gene. These results are consistent with the putative role of PKR as a tumor suppressor gene and in accordance with the idea that activation of the translational machinery may be one mechanism through which oncogenes exert their transforming function.

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Rosemary Jagus 7/29/95

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APPENDIX: Savinova O. and Jagus, R.: Deregulation of the interferon induced, dsRNA activated protein kinase, PKR, in breast carcinoma cells. Interferon and Cytokine Research, in press, 1995.

INTRODUCTION

This grant represents an innovative developmental and exploratory award to an investigator who had no previous background in human breast cancer, but who has a long history in the study of protein synthesis regulation and its role in the regulation of cell proliferation. The investigator seeks to investigate the possibility that aberrant regulation of cellular protein synthesis could underlie the loss of growth control in breast carcinoma cells through dysfunction of the dsRNA activated, eIF-2α-specific protein kinase, PKR. This kinase has been implicated as a tumor suppressor gene, because of its growth suppressive and translational inhibitory properties, as well as the ability of nonfunctional mutants of PKR to transform cells [1]. The role of PKR, in the loss of growth control in breast cancer cells has been investigated by a comparison of the expression and regulation of activity of PKR in normal breast and breast carcinoma cell lines. Furthermore, an evaluation of the role of PKR in the the estrogen responsiveness of breast carcinoma cell lines has been made by a comparison of PKR expression, and regulation of activity, in an estrogen responsive line treated with or without estrogen, or the estrogen antagonist, tamoxifen. Monoclonal antibodies are available to human PKR and have been used for the determination of PKR levels and phosphorylation (activation) state. The state of PKR activation has also be determined by an analysis of the phosphorylation state of its substrate, eIF-2α.

BODY

Four tasks were outlined in the **Statement of Work** in the application. These were:

1. Analysis of PKR expression in breast carcinoma tissue.
2. Analysis of PKR expression in breast carcinoma cell lines.
3. Analysis of the effect of estrogen antagonists on the regulation of PKR expression/activity in estrogen receptor positive breast carcinoma cell lines.
4. Analysis of PKR expression/activity in cell lines stably transformed with the human placental aromatase gene and effects of aromatase inhibitors.

Tasks 2 and 3 analyzing the characteristics of breast carcinoma cell lines have been completed. Over a range of cell densities, eIF-2α levels, PKR levels, PKR activation state and eIF-2α phosphorylation state has been monitored in two estrogen receptor positive cell lines, MCF7 and T47D, and two estrogen unresponsive cell lines, BT-20 and MDA-MB-468. These have been compared to the same parameters in a normal human breast cell line, Hs578 Bst. The results with breast carcinoma cell lines have also been compared to those obtained in the human HeLa fibroblast cell line, since much is known about the regulation of PKR expression/regulation in this system.

Levels of the protein synthesis initiation factor, eIF-2α are 2-3 fold higher in the human breast carcinoma cell lines compared to the normal human breast cell line and the HeLa cell line. To some extent this parallels the increased ribosome content (1.5-2 fold higher), although eIF-2α:ribosome ratios are higher in breast carcinoma cells compared to normal human breast cell lines. This is consistent with the increased protein synthetic activity necessary to sustain higher proliferation rates. It is also

consistent with the raised *c-myc* levels of breast carcinoma cells which increases the expression of eIF-2α [2]. In addition, levels of the eIF-2α-specific protein kinase, PKR, are also much higher in breast carcinoma cell lines compared with Hs578 Bst or HeLa cells. PKR levels in breast carcinoma cells are approximately equimolar with ribosomes, whereas in HeLa cells the equivalent level is 1 molecule of PKR for every 4-5 ribosomes [3]. In contrast to this, the phosphorylation state of eIF-2α is very low in the breast carcinoma cell lines compared to the normal human breast cell line Hs578 Bst and HeLa cells, even at high cell densities. In HeLa, PKR activity increases with increasing PKR levels. In HeLa and normal breast cells, eIF-2α phosphorylation state increases with increasing cell density. The high levels of PKR in breast carcinoma cells, in conjuction with low eIF-2α phosphorylation levels, suggests an inhibition of PKR activity in the breast carcinoma cell lines. Consistent with this hypothesis, treatment of cells with either α- or β-interferon increased PKR levels slightly, but did not result in higher steady state levels of eIF-2α phosphorylation. Similarly, incubation of breast carcinoma cells with dsRNA after interferon treatment did not result in higher steady state levels of eIF-2α phosphorylation. Interestingly, a combination of β-interferon and retinoic acid gave rise to increased levels of PKR mRNA, which was not reflected in increased levels of PKR.

In the estrogen receptor positive cell lines, estrogen removal or re-addition had no effects on PKR levels/activities. Furthermore, estrogen removal or re-addition had no effects on eIF-2α phosphorylation levels or phosphorylation state.

CONCLUSIONS

These results are consistent with the deregulation of PKR activity in breast carcinoma cell lines which could result from increased levels/activities of PKR inhibitory proteins or from inactivating mutations in the PKR gene. These results are consistent with the putative role of PKR as a tumor suppressor gene. The next step will be the analysis of PKR levels/activity in breast tumor samples. After confirmation of the results in tumors, the underlying mechanism(s) will be sought. Initially, analysis of the PKR gene will be pursued using a multiplex polymerase chain reaction amplification system that will reveal small deletions and insertions [4]. If the high PKR levels and low activities do not reflect nonfunctional mutations, it will be assumed that some element of the PKR regulatory cascade is dysfunctional. The initial assessment of this will be to assay for PKR inhibitory activity in breast carcinoma cell lines. In addition, levels of known cellular PKR inhibitors such as p58 [1,5] will be determined.

Knowledge of PKR regulation in breast carcinoma cells will allow us to pose some different questions on the interactions between hormones, growth factors, and cytokines. Furthermore, an understanding of PKR regulation in breast carcinoma cells should suggest what therapeutic agents might be effective in suppressin/reducing the growth of breast carcinomas.

Personnel funded by award: Olga Savinova, M.S. began work in the laboratory October, 1994.

Publications arising from funding: Savinova O. and Jagus, R.: Deregulation of the interferon induced, dsRNA activated protein kinase, PKR, in breast carcinoma cells. Interferon and Cytokine Research, 1995, in press.

REFERENCES

1. Jagus, R. and Gray, M. M. (1994) Proteins that interact with PKR. Biochimie **76**, 779-791.

2. Rosenwald, I. B., Rhoads, D. B., Callahan, L. D., Isselbacher, K. J., and Schmidt, E. V. (1993) Increased expression of eukaryotic translation factors eIF-4E and eIF-2α in response to growth induction by c-*myc*. PNAS **90**, 6175-6178.

3. Jeffrey, I., Kadereit, S., Meurs, E. F., Metzger, T., Schwemmle, M., Bachmann, M., Hovanessian, A. G., and Clemens, M. Localization of PKR in human and transfected mouse cells. Exptl. Cell Res. **218**, 17-27.

4. Runnebaum, I. B., Nagarajan, M., Bowman, M., Soto, D., and Sukumar, S. (1991) Mutations in p53 as potential molecular markers for human breast cancer. PNAS **88**, 10657-10661.

5. Lee, T. G., Tomita, J., Hovanessian, A. G., and Katze, M. G. (1992) Characterization and regulation of the 58 kDa cellular inhibitor of PKR. J. Biol. Chem. **267**, 14238-14243.

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DEREGULATION OF THE INTERFERON INDUCED, dsRNA ACTIVATED PROTEIN KINASE, PKR, in BREAST CARCINOMA CELLS

Olga Savinova and Rosemary Jagus, Center of Marine Biotechnology, UMBI, Baltimore, MD 21202

Levels of the protein synthesis initiation factor, eIF-2α are higher in the human breast carcinoma cell lines, MCF-7 and T-47D compared to the normal human breast cell line Hs578 Bst. This is consistent with the increased protein synthetic activity necessary to sustain higher proliferation rates. In addition, the phosphorylation state of eIF-2α is very low in the breast carcinoma cell lines compared to the normal human breast cell line Hs578 Bst and the mouse fibroblast cell line, NIH3T3, even at high cell densities. Surprisingly, levels of the eIF-2α-specific protein kinase, PKR, are also much higher in MCF-7 and T-47D cell lines compared with Hs578 Bst or 3T3 cells, suggesting an inhibition of PKR activity in the breast carcinoma cell lines. In support of this hypothesis, treatment of cells with either α- or β-interferon, although increasing PKR levels slightly, do not result in higher steady state levels of eIF-2α phosphorylation.

These results suggest that deregulation of PKR activity is occurring at some level in breast carcinoma cell lines. This deregulation could result from increased levels/ activities of cellular PKR inhibitory proteins, or from inactivating mutations in the PKR gene. These results are consistent with the putative role of PKR as a tumor suppressor gene and in accordance with the idea that activation of the translational machinery may be one mechanism through which oncogenes exert their transforming function.

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